Food Safety in Wine: Removal of Ochratoxin a in Contaminated White Wine Using Commercial Fining Agents

Authors : Antònio Inês, Davide Silva, Filipa Carvalho, Luís Filipe-Riberiro, Fernando M. Nunes, Luís Abrunhosa, Fernanda Cosme

Abstract : The presence of mycotoxins in foodstuff is a matter of concern for food safety. Mycotoxins are toxic secondary metabolites produced by certain molds, being ochratoxin A (OTA) one of the most relevant. Wines can also be contaminated with these toxicants. Several authors have demonstrated the presence of mycotoxins in wine, especially ochratoxin A. Its chemical structure is a dihydro-isocoumarin connected at the 7-carboxy group to a molecule of L-β-phenylalanine via an amide bond. As these toxicants can never be completely removed from the food chain, many countries have defined levels in food in order to attend health concerns. OTA contamination of wines might be a risk to consumer health, thus requiring treatments to achieve acceptable standards for human consumption. The maximum acceptable level of OTA in wines is 2.0 μg/kg according to the Commission regulation No. 1881/2006. Therefore, the aim of this work was to reduce OTA to safer levels using different fining agents, as well as their impact on white wine physicochemical characteristics. To evaluate their efficiency, 11 commercial fining agents (mineral, synthetic, animal and vegetable proteins) were used to get new approaches on OTA removal from white wine. Trials (including a control without addition of a fining agent) were performed in white wine artificially supplemented with OTA (10 μg/L). OTA analyses were performed after wine fining. Wine was centrifuged at 4000 rpm for 10 min and 1 mL of the supernatant was collected and added of an equal volume of acetonitrile/methanol/acetic acid (78:20:2 v/v/v). Also, the solid fractions obtained after fining, were centrifuged (4000 rpm, 15 min), the resulting supernatant discarded, and the pellet extracted with 1 mL of the above solution and 1 mL of H2O. OTA analysis was performed by HPLC with fluorescence detection. The most effective fining agent in removing OTA (80%) from white wine was a commercial formulation that contains gelatin, bentonite and activated carbon. Removals between 10-30% were obtained with potassium caseinate, yeast cell walls and pea protein. With bentonites, carboxymethylcellulose, polyvinylpolypyrrolidone and chitosan no considerable OTA removal was verified. Following, the effectiveness of seven commercial activated carbons was also evaluated and compared with the commercial formulation that contains gelatin, bentonite and activated carbon. The different activated carbons were applied at the concentration recommended by the manufacturer in order to evaluate their efficiency in reducing OTA levels. Trial and OTA analysis were performed as explained previously. The results showed that in white wine all activated carbons except one reduced 100% of OTA. The commercial formulation that contains gelatin, bentonite and activated carbon reduced only 73% of OTA concentration. These results may provide useful information for winemakers, namely for the selection of the most appropriate oenological product for OTA removal, reducing wine toxicity and simultaneously enhancing food safety and wine quality.

Keywords : wine, ota removal, food safety, fining
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FOOD SAFETY IN WINE: REMOVAL OF OCHRATOXIN A IN CONTAMINATED WHITE WINE USING COMMERCIAL FINING AGENTS

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The presence of mycotoxins in foodstuff is a matter of concern for food safety. Mycotoxins are toxic secondary metabolites produced by certain molds, being ochratoxin A (OTA) one of the most relevant. Wines can also be contaminated with these toxicants. Several authors have demonstrated the presence of mycotoxins in wine, especially ochratoxin A (OTA) [1]. Its chemical structure is a dihydro-isocoumarin connected at the 7-carboxy group to a molecule of L-β-phenylalanine via an amide bond. As these toxicants can never be completely removed from the food chain, many countries have defined levels in food in order to attend health concerns. OTA contamination of wines might be a risk to consumer health, thus requiring treatments to achieve acceptable standards for human consumption [2]. The maximum acceptable level of OTA in wines is 2.0 μg/kg according to the Commission regulation No. 1881/2006 [3]. Therefore, the aim of this work was to reduce OTA to safer levels using different fining agents, as well as their impact on white wine physicochemical characteristics. To evaluate their efficiency, 11 commercial fining agents (mineral, synthetic, animal and vegetable proteins) were used to get new approaches on OTA removal from white wine. Trials (including a control without addition of a fining agent) were performed in white wine artificially supplemented with OTA (10 µg/L). OTA analysis were performed after wine fining. Wine was centrifuged at 4000 rpm for 10 min and 1 mL of the supernatant was collected and added of an equal volume of acetonitrile/methanol/acetic acid (78:20:2 v/v/v). Also, the solid fractions obtained after fining, were centrifuged (4000 rpm, 15 min), the resulting supernatant discarded, and the pellet extracted with 1 mL of the above solution and 1 mL of H_2O. OTA analysis was performed by HPLC with fluorescence detection according to Abrunhosa and Venâncio [4]. The most effective fining agent in removing OTA (80%) from white wine was a commercial formulation that contains gelatine, bentonite and activated carbon. Removals between 10-30% were obtained with potassium caseinate, yeast cell walls and pea protein. With bentonites, carboxymethylcellulose, polyvinylpolypyrrolidone and chitosan no considerable OTA removal was verified. Following, the effectiveness of seven commercial activated carbons was also evaluated and compared with the commercial formulation that contains gelatine, bentonite and activated carbon. The different activated carbons were applied at the concentration recommended by the manufacturer in order to evaluate their efficiency in reducing OTA levels. Trial and OTA analysis were performed as explained previously. The results showed that in white wine all activated carbons except one reduced 100% of OTA. The commercial formulation that contains gelatine, bentonite and activated carbon (C8) reduced only 73% of OTA concentration. These results may provide useful information for winemakers, namely for the selection of the most appropriate oenological product

for OTA removal, reducing wine toxicity and simultaneously enhancing food safety and wine quality.

Acknowledgements

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Food Safety in Wine: Removal of Ochratoxin A in Contaminated White Wine Using Commercial Fining Agents

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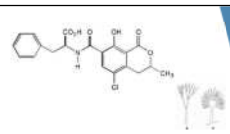

Introduction

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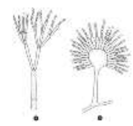
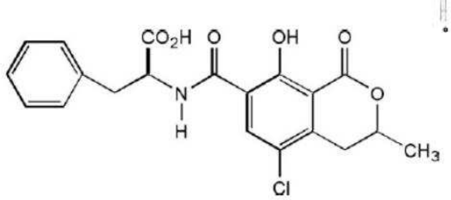
Mycotoxins

- ✓ The presence of mycotoxins in foodstuff is a matter of concern for food safety;
- ✓ Mycotoxins are toxic secondary metabolites produced by certain molds, being ochratoxin A (OTA) one of the most relevant;
- ✓ Wines can also be contaminated with these toxicants;
- ✓ In Europe, wine is estimated to be the second source, after cereals, of ochratoxin A (OTA).

Ochratoxin A





[illegible] et al., 2002

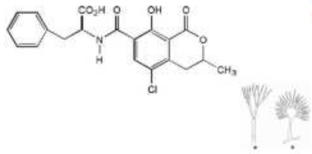
Its chemical structure is a dihydro-isocoumarin connected at the 7-carboxy group to a molecule of L-β-phenylalanine via an amide bond

Ochratoxin A (OTA)

- ✓ As these toxicants can never be completely removed from the food chain, many countries have defined levels in food in order to attend health concerns;
- ✓ OTA contamination of wines might be a risk to consumer health, thus requiring treatments to achieve acceptable standards for human consumption;
- ✓ The maximum acceptable level of OTA in wines is 2.0 μg/kg according to the Commission regulation No. 1881/2006 .

Ochratoxin A (OTA)

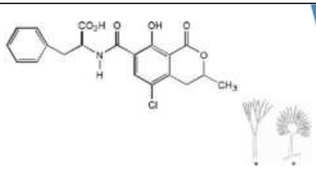
- ✓ Therefore, it is important to prevent and control their occurrence in wines.
- ✓ With the purpose to remove this toxin, several chemical, microbiological and physical methods were described in the literature.



Objective

Objective

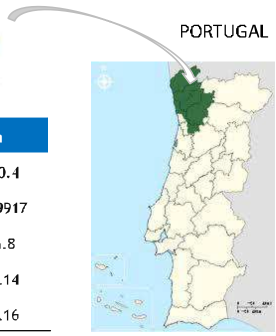
- ✓ The aim of this work was to reduce OTA to safer levels using different fining agents, as well as to understand the fining agents impact on white wine physicochemical characteristics.



Material and Methods

Wine samples

White wine from Vinho Verde Region	
Alcohol content (% v/v)	10.4
Specific gravity (20 °C) (g/mL)	0.9917
Titratable acidity (g/L tartaric acid)	6.8
pH	3.14
Volatil acidity (g/L acétic acid)	0.16



Fining experimental design

Commercial oenological products	
Sodium bentonite	B1
Calcium bentonite	B2
Potassium caseinate	C
Carboxymethylcellulose	CMC
Chitosan	Q
Polyvinylpolypyrrolidone	PVPP
Pea protein	PE
Mannoprotein	MP
Mixture composed by gelatin, bentonite and activated carbon	MIX

- ✓ Trials of 11 commercial oenological products with different characteristics
- ✓ Used at the average dose recommended by the manufacturer
- ✓ To assess their ability to remove OTA
- ✓ In artificially supplemented (10 µg/L) wine.

Fining experimental design

- ✓ 7 commercial activated carbons (C1-C7) and 1 mix composed by gelatin, bentonite and activated carbon (C8)
- ✓ Used at the maximum concentration recommended by the manufacture
- ✓ To get new approaches on OTA removal from white wine artificially supplemented with OTA at a final concentration of 10.0 µg/L.

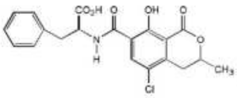
OTA Analysis

- ✓ After wine fining, the supernatant was centrifuged (4000 rpm; 10 min.)
- ✓ 1 mL of the supernatant was collected and added of an equal volume of acetonitrile/methanol/acetic acid (78:20:2 v/v/v).
- ✓ The solid fractions obtained after fining, were centrifuged (4000 rpm; 15 min) and the pellet extracted with 1 mL of the above solution and 1 mL of H_2O.

- ✓ OTA analysis was performed by HPLC with fluorescence detection according to Abrunhosa and Venâncio (2007).

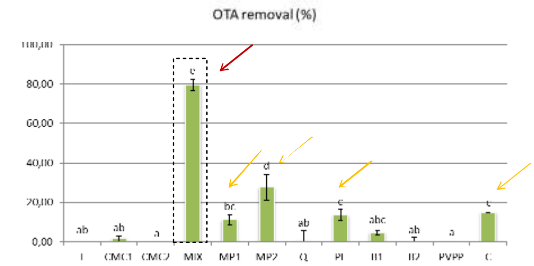
Wine quality parameters studied

- Analysis of conventional oenological parameters (OIV, 2012)
- Total phenols, non-flavonoid and flavonoids (Kramling e Singleton 1969)
- Browning potential (Singleton and Kramling, 1976)
- Colour at 420 nm (OIV, 2012)



Results and discussion

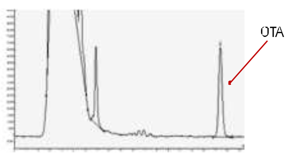
OTA removal (%) after white wine fining



- ✓ Most effective oenological product in removing OTA (80%) MIX (Gelatin, Bentonite and Activated Carbon).
- ✓ Removals between 10-30% obtained with Casein, Manoprotein and Pea protein.
- ✓ Bentonites, Carboxymethylcellulose, Polyvinylpolypyrrolidone and Chitosan do not removed considerable OTA.

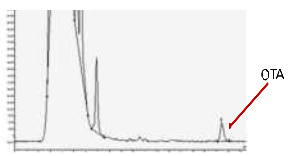
Chromatogram of OTA analysis

A



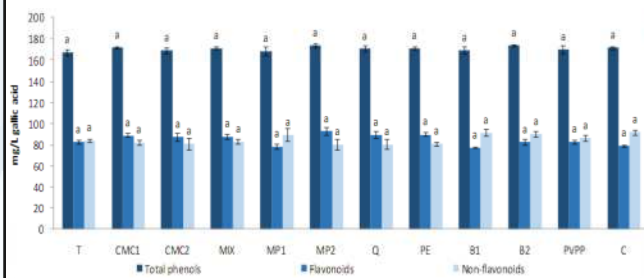
Chromatogram from the white wine without treatment

B



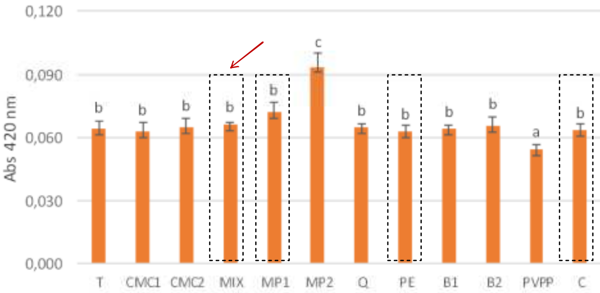
Chromatogram from the white wine treated with commercial formulation that contains activated carbon

Impact of fining on white wine phenolic compounds



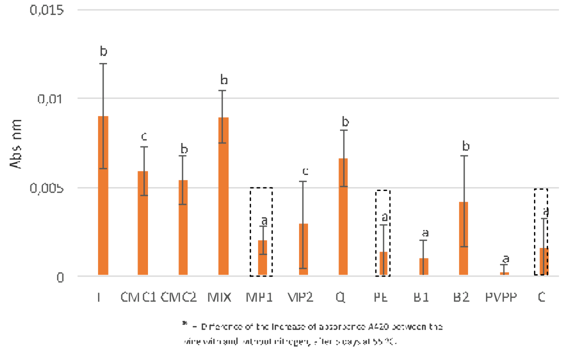
After fining, in white wine, total phenols, non-flavonoids and flavonoids did not decrease significantly.

Impact of fining on white wine color



The wine color was not altered by the application of MIX, MP1, PE and C. The oenological products that better removed OTA.

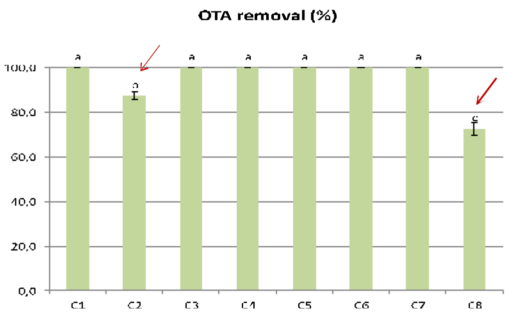
Impact of fining on white wine browning potential



The oenological products that performed better in removing OTA:

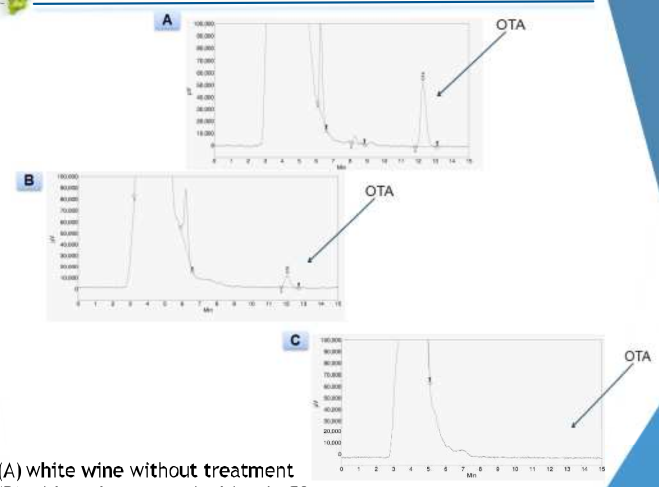
- ✓ The PE, MP1 and C were effective in reducing wine browning potential
- ✓ The MIX was not efficient in reducing wine browning potential.

OTA removal (%) after white wine fining with activated carbon



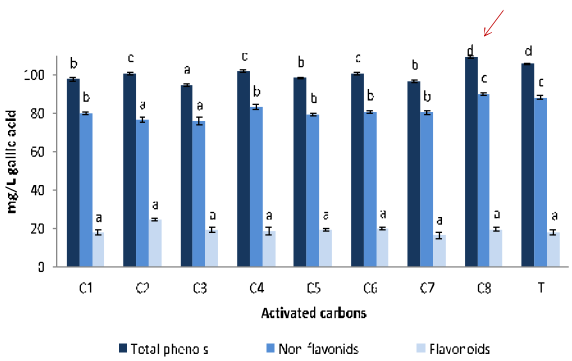
- ✓ Activated carbons except one reduced 100% of OTA.
- ✓ Mixture composed by gelatine, bentonite and activated carbon (C8) reduced 73% of OTA .

Chromatogram of OTA analysis



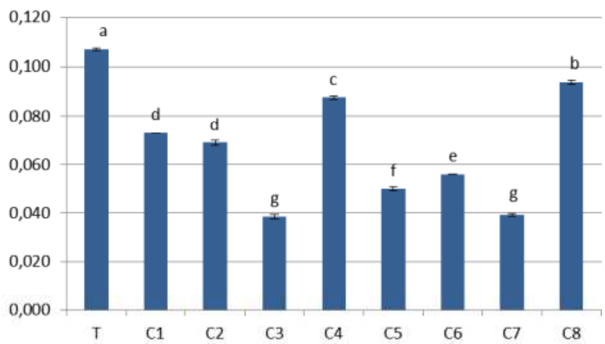
(A) white wine without treatment
(B) white wine treated with mix C8
(C) white wine treated with activated carbon C3

Impact of activated carbon on wine phenolic compounds

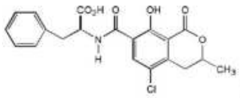


After fining, total phenols and non-flavonoids decreased significantly with exception of carbon C8, in white wine.

Impact of activates carbon on white wine color



The wine color was significantly altered by the application of activates carbons.



Conclusions

Final considerations

These results may provide useful information for winemakers:

- ✓ For the selection of the most appropriate enological product for OTA removal.
- ✓ Reducing the toxicity and simultaneously enhancing food safety and wine quality.

Thank you for your attention

This work was funded by FEDER funds through the COMPETE and by national funds through FCT, Ref. FCOMP-01-0124-FEDER-028029 and PTDC/AGR-TEC/3900/2012, respectively. This work was also funded by IBB/CGB-UTAD and Chemical Research Centre of Vila Real (CQ-VR). Additional thanks to SAI Lda., AEB Bioquímica Portuguesa, S. A. and Enartis companies for providing fining agents. Luis Abrunhosa received support through grant SFRH/BPD/43922/2008 from FCT.

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